a strong acid such as trichloroacetic acid into the solution (generally from a storage module) causes the longer probe to precipitate, while the smaller cleaved fragments remain in solution. The use of frits or filters can to remove the precipitate, and the cleaved probe sequences can be quantitated.

[0209] In a preferred embodiment, the scissile probe contains both an ETM and an affinity binding ligand or moiety, such that an affinity support is used to carry out the separation. In this embodiment, it is important that the ETM used for detection is not on the same probe sequence that contains the affinity moiety, such that removal of the uncleaved probe, and the cleaved probe containing the affinity moiety, does not remove all the detectable ETMs. Alternatively, the scissile probe may not contain a covalently attached ETM, but just an affinity label. Suitable affinity moieties include, but are not limited to, biotin, avidin, streptavidin, lectins, haptens, antibodies, etc. The binding partner of the affinity moiety is attached to a solid support (again, either an internal surface of the device or to beads trapped within the device) and the flow of the sample past this support is used to pull out the uncleaved probes, as is known in the art. The cleaved probe sequences, which do not contain the affinity moiety, remain insolution and then can be detected as outlined below.

[0210] In a preferred embodiment, similar to the above embodiment, a separation sequence of nucleic acid is included in the scissile probe, which is not cleaved during the reaction. A nucleic acid complementary to the separation sequence is attached to a solid support and serves as a catcher sequence. Preferably, the separation sequence is added to the scissile probes, and is not recognized by the target sequence, such that a generalized catcher sequence may be utilized in a variety of assays.

[0211] In a preferred embodiment, the uncleaved probe is neutralized by the addition of a substantially complementary neutralization nucleic acid, generally from a storage module. This is particularly useful in embodiments utilizing capture sequences, separation sequences, and one-step systems, as the complement to a probe containing capture sequences forms hybridization complexes that are more stable due to its length than the cleaved probe sequence:detection probe complex. As will be appreciated by those in the art, complete removal of the uncleaved probe is not required, since detection is based on electron transfer through nucleic acid; rather, what is important is that the uncleaved probe is not available for binding to a detection electrode probe specific for cleaved sequences. Thus, in one embodiment, this step occurs in the detection module and the neutralization nucleic acid is a detection probe on the surface of the electrode, at a separate "address", such that the signal from the neutralization hybridization complex does not contribute to the signal of the cleaved fragments. Alternatively, the neutralization nucleic acid may be attached to a solid support; the sample flowed past the neutralization surface to quench the reaction, and thus do not enter the detection module.

[0212] After removal or neutralization of the uncleaved probe, detection proceeds via the addition of the cleaved probe sequences to the detection module, as outlined below, which can utilize either "mechanism-1" or "mechanism-2" systems.

[0213] In a preferred embodiment, no higher order probes are used, and detection is based on the probe sequence(s) of the primary primer. In a preferred embodiment, at least one, and preferably more, secondary probes (also referred to herein as secondary primers) are used. The secondary scissile probes may be added to the reaction in several ways. It is important that the secondary scissile probes be prevented from hybridizing to the uncleaved primary probes, as this results in the generation of false positive signal. In a preferred embodiment, the primary and secondary probes are bound to solid supports. It is only upon hybridization of the primary probes with the target, resulting in cleavage and release of primary probe sequences from the bead, that the now diffusible primary probe sequences may bind to the secondary probes. In turn, the primary probe sequences serve as targets for the secondary scissile probes, resulting in cleavage and release of secondary probe sequences. In an alternate embodiment, the complete reaction is done in solution. In this embodiment, the primary probes are added, the reaction is allowed to proceed for some period of time, and the uncleaved primary scissile probes are removed, as outlined above. The secondary probes are then added, and the reaction proceeds. The secondary uncleaved probes are then removed, and the cleaved sequences are detected as is generally outlined herein. In a preferred embodiment, at least one, and preferably more, tertiary probes are used. The tertiary scissile probes may be added to the reaction in several ways. It is important that the tertiary scissile probes be prevented from hybridizing to the uncleaved secondary probes, as this results in the generation of false positive signal. These methods are generally done as outlined above. Similarly, quaternary probes can be used as above.

[0214] Thus, CPT requires, again in no particular order, a first CPT primer comprising a first probe sequence, a scissile linkage and a second probe sequence; and a cleavage agent.

[0215] In this manner, CPT results in the generation of a large amount of cleaved primers, which then can be detected as outlined below.

[0216] In a preferred embodiment, the signal amplification technique is a "sandwich" assay, as is generally described in U.S. Ser. No. 60/073,011 and in U.S. Pat. Nos. 5,681,702, 5,597,909, 5,545,730, 5,594,117, 5,591,584, 5,571,670, 5,580,731, 5,571,670, 5,591,584, 5,624,802, 5,635,352, 5,594,118, 5,359,100, 5,124,246 and 5,681,697, all of which are hereby incorporated by reference. Although sandwich assays do not result in the alteration of primers, sandwich assays can be considered signal amplification techniques since multiple signals (i.e. label probes) are bound to a single target, resulting in the amplification of the signal. Sandwich assays are used when the target sequence comprises little or no labels; that is, when a secondary probe, comprising the labels, is used to generate the signal.

[0217] As discussed herein, it should be noted that the sandwich assays can be used for the detection of primary target sequences (e.g. from a patient sample), or as a method to detect the product of an amplification reaction as outlined above; thus for example, any of the newly synthesized strands outlined above, for example using PCR, LCR, NASBA, SDA, etc., may be used as the "target sequence" in a sandwich assay.

[0218] Generally, sandwich signal amplification techniques may be described as follows. The reactions described below can occur either in the reaction module, with subsequent transfer to the detection module for detection, or in the